

**METHOD OF PREPARING BIOLOGICAL MATERIALS FOR
CRYOPRESERVATION USING PRE-CHILLED PROTECTANT**

ABSTRACT OF THE DISCLOSURE

A method of preparing biological materials for cryopreservation is presented.

5 The method lessens the amount of heat released by a cryoprotectant during a latent
heat phase by freezing the protectant, thawing the protectant, and treating biologically
active materials with the thawed protectant. First, the protectant is frozen to induce
an irreversible phase change, along with an irreversible release of energy. After this
phase change has occurred, the protectant is thawed and used to treat viable cells or
10 other biologically active material about to undergo freezing. The thawed protectant
within the biologically active cells has a reduced endothermic reaction upon
subsequent freezing. In one embodiment, freezing the biological material includes
immersing the biological material in cooling fluid and circulating the cooling fluid
past the biological material at a substantially constant, pre-determined velocity and
15 temperature such that the biological material is vitrified, thus minimizing the
formation of stress fractures in cell membranes. The protectant may be propylene
glycol, glycerol, DMSO, or other suitable protectants.